

RESEARCH PAPER

Isolation and PGPR characterization of cadmium resistant strains RCP 1 in cadmium contaminated soil

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Resistance and tolerance are arbitrarily defined terms frequently interchangeable and after based on whether a given isolate can grow in the presence of selected heavy metal concentration in laboratory media. Total bacterial strains were isolated and screened on the basis of their heavy metal tolerance ability for cadmium and their PGPR activity. It was estimated that they have good tolerance power for cadmium, the heavy metals and are plant growth promoting rhizobacteria. If we study morphological aspects we can say that Gram negative rods are more efficient in tolerating heavy metals. By performing different PGPR test it was proved that the isolated strains have PGPR activity can be used as biopesticides and biofertilizers. At pH 7 and 28°C temperature strain RCP 1 was resistant to heavy metal but at pH 9, the growth was inhibited in presence of heavy metal. There were high growth rate of strain RCP 1 at 28°C. Strain RCP 1 grew at neutral and alkaline pH also, but in presence of heavy metal growth was increased. In presence of heavy metal, strain RCP 1 increased the root and shoot length as well as fresh and dry weight also. So, the microbes isolated from RCP 1 that must have good tolerance power and developed resistance mechanism for their survival and thus, can be helpful in bioremediation.

Key words : Rhizobacteria, Tolerance, Resistance, PGPR, Cadmium

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INTRODUCTION

Heavy metal is natural constituent of the earth crust and is present in varying concentration in all ecosystems. Heavy metal is chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Copper, chromium, cadmium are known to be the most commonly metal used and more widespread contaminants of their environment (Patterson, 1977; Aksu, 1998 and Doenmez and Aksu, 2001). Heavy metal could have long term hazardous impact on health of soil micro-organism and adverse influence on soil biological process Khan *et al.* (2009). Cadmium is one of the most toxic pollutants of

the surface soil layer, released into the environment by mining and smelting activities, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, land application of sewage sludge and burning of fossil fuels (Tang *et al.*, 2006). Cadmium is heavy metal of no nutrition function, it is a natural element in the earth crust and usually found as a mineral combined with other element such as oxygen (cadmium oxide), chlorine (cadmium chloride), sulfur (cadmium sulfate), and is a toxic agent to micro-organism, but some are microbial strain resistant to the metal. The present investigation was carried out to isolate and functional characterization

of cadmium resistant strains RCP 1 and its effect on chaulai plant in cadmium contaminated soil.

RESEARCH METHODOLOGY

Collection of soil samples :

Soil sample was collected from the rhizospheric soil of plants from Panki power house. The collected soil samples and strains were stored at 4°C. The details of soil samples collected from rhizosphere are:

Sr. No.	Soil sample	Source	Plant	Colour	Text-ure	Arran-gement	pH
1.	RCP	Rhizo-sphere	<i>Calotropis procera</i> (Madar)	Dark brown	Rough	Clumpy	9

Isolation of sample :

Soil sample (2 g) was grounded and suspended in 20 ml sterile water. Then isolation of the sample was done by serially diluting it and 0.1 ml of diluted suspension was placed on nutrient agar medium and spread evenly on solid plates. The plates were incubated at suitable temperature for different time intervals. The screenings of the strains were done on the basis of their tolerance power with cadmium concentration (Ahmad *et al.*, 2008).

Heavy metal tolerance :

Stock solution of cadmium was made (1g in 25 ml distilled water). Different concentration of cadmium in media was pipetted and tolerance power of the strains was determined (Mark, 1987).

Identification of bacteria :

Bacterial colonies were identified by Gram staining, starch hydrolysis test, gelatin hydrolysis test, indole test, methyl red test, voges porskauer test, citrate utilization test, triple sugar iron agar test, urease test, catalase test and H₂S production tests were performed (Verma, 2013).

Plant growth promoting activity :

Phosphate solubilization :

The phosphate solubilization by the rhizospheric

microbes was detected and estimated by the method of Tank and Saraf (2010).

HCN production :

HCN production was performed by the method described by Bakkar *et al.* (1987).

Siderophore production:

Siderophore production by selected bacterial cultures was detected (Schwyn and Neillands, 1987). Both qualitative and quantitative determination of siderophore was done.

Screening of physiological characterization of bacterial strains :

Temperature and pH were determined for screening of physiological characterization of bacterial strains.

RESEARCH FINDINGS AND ANALYSIS

Soil microbial population is known to play a key role in maintaining soil fertility by recycling of plant nutrients and also for removal of metals in contaminated soils. In this context various approaches have been used to study microbial activities in soil. As it is difficult to elucidate soil microbial activity using single monitoring approach, therefore, for a better insight and complete depiction of soil microbial situation, different approaches need to be used. In present investigation total of 4 bacterial isolates were isolated from rhizospheric soil of *Calotropis procera* (Madar) from Panki power house and tentatively identified on the basis of morphological and biochemical characterization. All bacterial isolates belonged to the gram negative groups.

Heavy metal tolerance :

Among all these strains maximum tolerance for cadmium was shown by strain RCP 1 as shown in Table 1.

Characterization of bacteria :

Specific soil strains were isolated from soil sample

Table 1 : Heavy metal tolerance of soil micro-organism						
Sr. No.	Rhizosphere	Strains	Cd (µg/ml)	Incubation time (h)	Incubation temp.	Media
1.	<i>Calotropis procera</i>	RCP 1	1400	18-24	28°C	Nutrient agar
2.	(Madar)	RCP 2	600	18-24	28°C	
3.		RCP 3	700	18-24	28°C	
4.		RCP 4	490	18-24	28°C	

along with concentration of cadmium on nutrient agar medium. These isolates were tentatively identified on the basis of their staining, behaviour, morphological and cultural characteristics as shown in Table 2.

Biochemical characterization of all isolated strains PGPR characterization :

Considerable amount of work has been generated in last few decades on plant growth promoting rhizobacteria to remove heavy metal pollutant (Glick, 2003). In the present investigation 4 distinct bacterial strains were screened as shown in Table 3. Some isolates were solubilized in phosphate. Maximum amount of phosphate was solubilized by strains RCP 1 on Pikovaskaya's agar media.

Out of four bacterial strains RCP 1 and RCP 2 showed HCN production. Rhizobacteria use the root exudates in rhizosphere for growth and development. Root

exudates have various type of molecule eg. growth factor, amino acids, alkaloids. Glycine is used by microbes as carbon source in rhizosphere through root exudates. Here cyanisation of glycine takes place due to which HCN production takes place. Cyanogenesis by rhizobacteria in the rhizosphere can adversely affect plant growth. HCN produced in the rhizosphere of seedling by *Pseudomonas* is a potentially and environmentally compatible mechanism in for biological control of weeds as shown in Table 4 (Baker, 1981). Maximum amount of fluorescence was shown by strain RCP 1. Siderophores are the iron chelators that complex with iron and make them readily available to plant root surfaces. Siderophore production increases with amount of organic substrates added to the soil. Its production can be detected by using chrom- Azurol S agar medium as shown in Table 4.

Only strain RCP 1 showed indole acetic acid production. IAA (Auxin) is a growth hormone which

Table 2 : Morphological characterization of all isolated strains

Strains	Gram staining
RCP 1	Gram negative, rod shaped, fluorescent
RCP 2	Gram negative, rod shaped.
RCP 3	Gram negative, rod shaped, fluorescent.
RCP 4	Gram negative, rod shaped.

Table 3: Biochemical characterization of all isolated strains

Sr. No.	Strains	Starch hydrolysis	Gelatinase	Indole production	M R	V P	Citrate utilization	Triple sugar iron agar test	Urease	Catalase	H ₂ S production
1.	RCP 1	+	+	+	+	+	+	+	-	+	-
2.	RCP 2	-	-	-	+	+	-	+	-	+	-
3.	RCP 3	-	-	-	-	-	-	+	-	+	-
4.	RCP 4	-	-	-	+	+	-	-	-	+	-

Table 4: PGPR Characterization of soil bacteria

Sr.No.	Strains	Phosphate solubilization		HCN production	Siderophore		Indole acetic acid production
		(+/-)	Diameter (cm)		Qualitative	Quantitative	
1.	RCP 1	+	2.6	+	+	2.7	0.412
2.	RCP 2	-	-	+	-	-	-
3.	RCP 3	-	-	-	+	0.45	-
4.	RCP 4	+	1.3	-	-	-	-

Table 5: Heavy metal tolerance and PGPR characterization of strain RCP 1

Sr.No.	Strain	Cd concentration (µg/ml)	Phosphate solubilization		HCN production	Siderophore		Indole acetic acid production
			(+/-)	Diameter (cm)		Qualitative	Quantitative	
1.	RCP 1	1400	+	2.6	+	+	2.7	0.412

induces the growth of an organism in low concentration. It is produced by fungi and bacteria in culture medium as well as infected plant tissue. In host parasite interaction level of auxin get changed. L-tryptophan is precursor of IAA, therefore, for production of IAA *in vitro* L-tryptophan should be amended in culture medium inoculated with test organism as shown in Table 4.

Heavy metal tolerance and PGPR characterization of strain RCP 1 :

Screening of the microbial strains was done on the basis of heavy metal tolerance character and their PGPR characteristics and its functional characterization was done using agronomical parameters of chaulai plant (*Amaranthus tricolor* L.). The selected strain was RCP 1. The isolates were found to exhibit heavy metal resistance characteristics and also showed the production of phosphate solubilization, HCN production, siderophore production and indole acetic acid production as shown in Table 5.

Physiological characterization of bacterial strain RCP 1:

Physiological characterization of bacterial strain RCP 1 in the absence of cadmium at different temperatures:

The growth curve of strain RCP 1 in the absence of cadmium (Fig. 1) showed that optimum temperature for strain RCP 1 was 28°C. At 37°C in the absence of cadmium (Fig. 2) the growth rate was very slow. At 45°C in the absence of cadmium (Fig. 2) no growth has taken place. At 28°C, in the absence of cadmium (Fig. 2) the growth of strain RCP 1 was increased (8.94%). Strain RCP 1 gave green fluorescent pigment within 12 h in absence of cadmium. Temperature influences the activity of bacterial enzymes. Enzymes are most active at

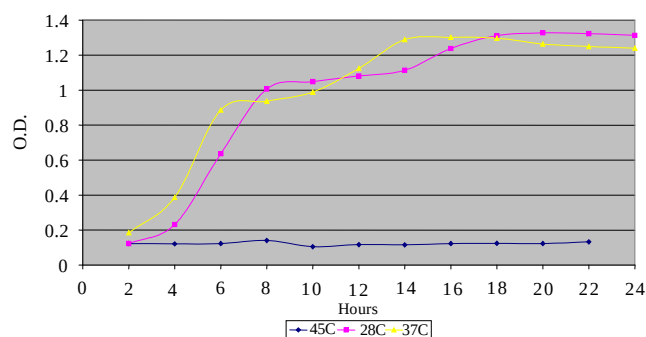


Fig. 1: Growth curve of strains in absence of cadmium at different temperature (28, 37 and 45°C) for strain RCP 1

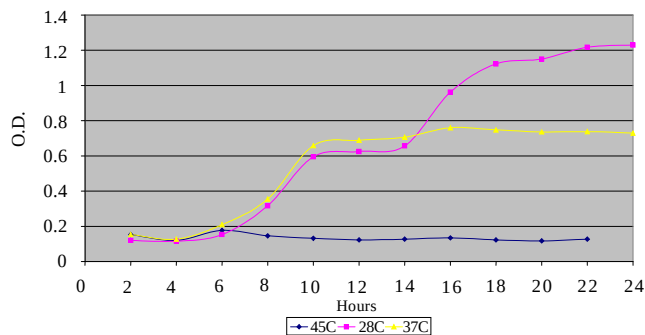


Fig. 2: Growth curve of strains in presence of cadmium at different temperature (28, 37 and 45°C) for strain RCP 1

optimum temperature and enzymatic reaction proceeds at maximum speeds. Below minimum temperature and above maximum temperature enzymes become inactive (Rai *et al.*,1981; Macaskie and Dean,1989; Huang *et al.*,1990; Avery and Tobin,1993 and Brady *et al.*,1994).

Physiological characterization of bacterial strain RCP1 in the presence of cadmium at different temperature :

The growth curve of strain RCP 1 in presence of cadmium (Fig. 2) showed that optimum temperature for strain RCP 1 was 28°C. At 37°C in the presence of cadmium (Fig. 2) the growth rate was very slow. At 45°C in the presence of cadmium (Fig. 2) no growth has taken place. In presence of cadmium, the growth of strain RCP 1 was decreased in comparison to growth appeared in absence of cadmium. At 28°C, in the presence of cadmium (Fig. 2) the growth of strain RCP 1 was decreased (8.20%) and showed a long lag phase in the presence of cadmium. Similar results have been reported earlier by Pal *et al.* (2004) and Edward Raja *et al.* (2006). Strain RCP 1 gave green fluorescent pigment within 12 h in presence of cadmium.

High concentration of cadmium metal ions is poison in the environment stopping biological metabolic activity and microbial growth. Above the optimal temperature, the growth rate decrease and thermal death may occur (Veglio *et al.*, 1997; Liu *et al.*, 2004 and Malik, 2004).

Growth curve of strains in absence of cadmium at different pH (5, 7 and 9) :

Optimum pH for strain RCP 1 was 7 (Fig. 3) but at pH 9, the growth of strain RCP 1 was equal in the absence of cadmium. So strain RCP 1 required either neutral or alkali media for its growth because at pH 5,

no growth has taken place in the absence of cadmium (Fig. 3). In absence of cadmium at pH 7, the growth of strain RCP 1 increased (12.71%) in comparison to growth occurred in presence of cadmium concentration. Strain RCP 1 gave green fluorescent pigment at pH 9 within 24 h in absence of cadmium.

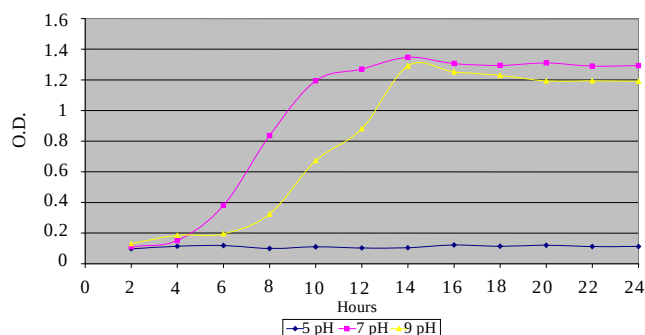


Fig. 3: Growth curve of strains in presence of cadmium at different pH (5, 7 and 9)

Growth curve of strains in presence of cadmium at different pH (5, 7 and 9) :

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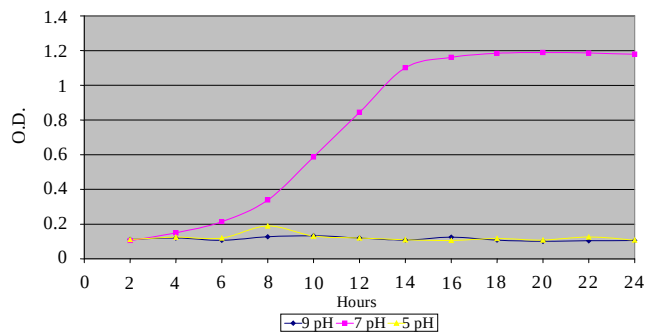


Fig. 4: Growth curve of strains in presence of cadmium at different pH (5, 7 and 9)

growth has taken place in the presence of cadmium (Fig. 4). In presence of cadmium concentration the growth of strain RCP 1 was decreased (11.27%). But at pH 9, the growth of strain RCP 1 was decreased (89.15%) in presence of cadmium (Fig. 4). At pH 7, strain RCP 1 showed a long lag phase in the presence of cadmium. The hydrogen ion concentration of an organism environment has the maximum influence on bacterial growth. It limits the synthesis of bacterial enzymes responsible for synthesizing the new protoplasm. Each micro-organism has its optimum pH. If the other factors such media composition, temperature or osmotic pressures vary, the pH will also vary (Brown *et al.*, 1987).

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